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PREVALENCE OF BOVINE TUBERCULOSIS IN DAIRY CATTLE OF JAIPUR, RAJASTHAN, INDIA

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Abstract– Tuberculosis in cattle is a chronic debilitating infectious disease caused by *Mycobacterium bovis,* an intracellular acid-fast bacterium. The diseases have become prevalent infection in most of the developing countries and causes considerable economic losses to producers of livestock, particularly in dairy cows. This study was conducted to evaluate the prevalence of tuberculosis in cattle in Jaipur. A total of 200 serum samples were collected from cattle of a gaushala in Jaipur and subjected to ELISA and single intradermal tuberculin test. overall prevalence obtained was 1.5% in cattle. Individual test wise, 6 (3%) and 9 (4.5%) animals were found positive to SITT and ELISA, respectively. Higher prevalence was observed in age group above 5 years as 3.65% and 5.11% by SITT and ELISA, respectively. Also, the prevalence was found higher in crossbred cattle as 3.45 and 6.89 by SITT and ELISA, respectively. Comparison of results for SITT and ELISA tests was found statistically non-significant ($p > 0.05$) with respect to age and breed. The kappa value to know the agreement between SITT and ELISA test was 0.38. It shows a poor agreement between both the tests for tuberculosis in cattle. The prevalence of bovine tuberculosis in milch animals signifies potential health risk for human and animals. The findings are required further confirmation with large scale surveillance of all susceptible animals.

INTRODUCTION

Bovine tuberculosis (BTB) is an infectious, chronic and contagious disease affecting livestock, wildlife and humans. It is caused by *Mycobacterium bovis*. It is a disease which causes a great economic disturbance and a significant public health risk in several countries in the world (Pollock *et al*., 2005). The public health concern is particularly significant in developing countries due to lack of appropriate preventive measures (Etter *et al*., 2006).

Especially in developing countries including India, Bovine tuberculosis (BTB) has been identified as one of the eight worldwide neglected zoonoses which needs more attention (WHO, 2012). It is present in many developing countries which have inadequacy or unavailability of surveillance and control measures (Cosivi *et al*., 1998). India is one of these countries, in which many epidemiological and public health aspects of the infection remain largely unknown.

More than 50 million cattle are infected worldwide with BTB. It results in economic losses of approximately $ 3 billion annually (Asford *et al*., 2001). Reports from various countries showed prevalence ranging from 1.65 to 24.3% for BTB (Boukary *et al*., 2011). Bovine tuberculosis leads to a great economic loss to the farmers. This is a contagious disease therefore spreads among the healthy animals from the infected ones. The BTB infection leads to a decrease in milk production upto 10-20%. It also results in loss of weight and a reduction of fertility. In addition, export of meat of infectious animal is also restricted in the countries where BTB is controlled (Brasil, 2006; Collins, 2006).

Meaningful success in the control of this infection

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can be done by a reliable means of diagnosis that can identify all the individuals transmitting the disease (WOAH, 2022). Traditional test-and-slaughter policies based on tuberculin testing have not been fully successful so that additional diagnostic tests are required to diagnose BTB (Pollock *et al*., 2005).

Isolation of *M. bovis* is the "Gold standard" for BTB diagnosis. However, much time required for the isolation and biochemical identification, is one of its critical points. It may require more than twelve weeks completing the final diagnosis, and also having low sensitivity (Collins *et al*., 1994). According to WOAH also, the gold standard test for diagnosis of bovine tuberculosis is culture and isolation of bacteria but as it is a time-consuming cumbersome process, rarely available in resource poor countries and consequently, indirect testing of mycobacterium antibodies in serum is applied for tuberculosis screening.

The intradermal tuberculin testis the official test for bovine tuberculosis in most of the countries and it has been used for routine field detection of infected animals since nearly a century ago (Monaghan *et al*., 1994).

Till date, sufficient data about actual prevalence of tuberculosis in this region is not available. The current study describes the prevalence and distribution of bovine tuberculosis with respect to age and sex in milch animals of Jaipur region.

MATERIALS AND METHODS

A total of 200 samples were collected for this study, from Hingoniya gaushala (26.82603'N, 75.94302'E) of Jaipur, comprising samples from milking cattle, particulars in terms of age and breed were collected as shown in Table 1 and 2. All samples were subjected to ELISA tests for diagnosis of bovine tuberculosis and the same 200 animals were also tested by single intradermal tuberculin test.

For single intradermal tuberculin test, bovine tuberculosis PPD procured from Biological Products Division (B.P. Division), Indian Veterinary Research Institute (IVRI), Izatnagar and stored at 4°C. Single intradermal tuberculin test was performed as per WOAH Terrestrial Manual (2022). The animal was considered as positive if the increase in the skin fold thickness was 4 mm or more, negative if 2 mm or less and it is considered as doubtful or inconclusive if the increase in skin fold thickness was between 2 to 4 mm.

Table 2. Breed-wise distribution of cattle

Species (No. of samples)	Breed	No. of samples
Cattle (200)	Native	142
	Crossbred	58

About 5 ml blood was collected aseptically from each cow. Serum is separated and stored at -20°C for further analysis. Collected serum samples were subjected to ELISA test.

ELISA was performed by using BIONOTE BTB Ab ELISA 2.0kit manufactured by BioNote Inc. The test was performed as per protocol outline in the user manual supplied with the kit.

Statistical analysis

The results obtained from SITT and ELISA tests were statistically analyzed for kappa values as per Thrusfield (2005). The degree of association between risk factors and the prevalence was also assessed by Pearson Chi-square test using SPSS 16 statistical software.

RESULTS AND DISCUSSION

Prevalence by Single Intradermal Tuberculin Test

In the present study, total 200 milking cattle were screened by single intradermal tuberculin test. Out of 200, only 6 (3.0 per cent) animals showed positive reaction, while 11 (5.5 per cent) animals were doubtful reactors. The prevalence of bovine tuberculosis was found 3.0% in the present study.

The present findings are in agreement with the reports of Boukary *et al*. (2011) who reported 3.6% overall apparent individual animal prevalence in cattle in Torodi. Katale *et al*. (2013) also recorded 2.4% prevalence in cattle in Tanzania, Pandey *et al*. (2013) reported 2.6% prevalence in cattle in southern Zambia, Asante-Poku *et al*. (2014) in Ghana reported

Table 1. Age-wise distribution of cattle

Species (No. of samples)	Age-group(yrs)	No. of samples
Cattle (200)	Below 5 Years	63
	Above 5 years	**137**

2.48% individual animal prevalence in cattle. Phaniraja *et al*. (2010) also recorded 2.4% prevalence in cattle in and around Bangalore, India.

In the present study, the prevalence of tuberculosis among cattle was higher than that reported by Ganesan (2013) in Tamil Nadu (1.68%), Sarker *et al*. (2015) in Mymensingh district in Bangladesh (2.34%), Mugambi *et al*. (2016) in eastern zone (EZ) of Tanzania (2.37%) but lower than that recorded by Omer *et al*. (2001) reported 14.5% prevalence in cattle in Asmara, Eritrea, Mukherjee (2006) in northern India (15.76%), Thakur *et al*. (2010) in Dangme Himachal Pradesh (14.31%) and Thakur *et al*. (2015) in organized dairy farm-Bareilly, dairy farm-Mukteshwar and Gaushala-Bareilly (13.12%).

Seroprevalence by ELISA testing

In the present study, out of 200 serum samples, 9 samples were found positive. The seroprevalence of bovine tuberculosis was 4.50% by ELISA testing.

The present findings are in agreement with the reports of Akalu (2017) who surveyed and found 3.5 to 5.2% prevalence in cattle in slaughter houses in Ethiopia. Awah-Ndukum *et al*. (2012) also recorded 4.67% prevalence in cattle in the highlands of Cameroon and Noorrahim *et al*. (2015) reported 4.33% prevalence in cattle in three tehsils of District Charsadda of Pakistan. The present findings are in agreement with the reports of Bhanurekha *et al*. (2014) who reported 4.48% seroprevalence in cattle in Tamil Nadu.

In this study, the seroprevalence of tuberculosis among cattle was higher than that reported by Didugu *et al*. (2016) in Krishna district, Andhra Pradesh (0.87%), Dutta *et al*. (2016) in western India (0.23-1.55%) and Neeraja *et al*. (2014) in Bangalore, India (0%) and but lower than that recorded by Thakur *et al*. (2015) in Uttar Pradesh (25.65), Rani *et al*. (2018) in Haryana (69%) and Das *et al*. (2017) in four districts of Gangetic delta region of West Bengal, India (62.4%).

Epidemiological pattern of tuberculosis in cattle

The epidemiological pattern of tuberculosis was estimated for which records on age and breed of animal were collected for cattle.

Age

In the present study, cattle were divided in 2 age groups *i.e.*, below 5 years and above 5 years. The higher prevalence was observed in above 5 years age group as 3.65% and 5.11% by SIT and ELISA, respectively; (Table 3) followed by below 5 years age group as 1.59% and 3.17% by SITT and ELISA, respectively. It indicates that adult age group of cattle are more susceptible than younger age group for bovine tuberculosis.

The study is in agreement with Bhanurekha *et al*. (2014) who found higher prevalence in cattle of above 5 years of age in Tamil Nadu. These findings are also in agreement with the observations of Konch *et al*. (2017) who reported higher seroprevalence in cattle of 6-9 years of age (40%) in Assam.

Results are also in agreement with the observation of Mahmud *et al*. (2014) who observed that prevalence was relatively high in older cattle in Sirajganj district of Bangladesh. Griffin *et al*. (1996) in a cross-sectional study carried out in Ireland

Table 3. Prevalence of tuberculosis in cattle in relation to age and breed by SITT test

Variables	Category	No. of animals tested	No. of animals positive (%)	Chi-square value	p-value
Age	Below 5 years	63	1 (1.59)	0.6307	0.427
	Above 5 years	137	5 (3.65)		
Breed	Cross bred	58	2 (3.45)	0.0564	0.812
	Indigenous	142	4 (2.82)		

Table 4. Prevalence of tuberculosis in cattle in relation to age and breed by ELISA test

Variables	Category	No. of animals tested	No. of animals positive (%)	Chi-square value	p-value
Age	Below 5 years	63	2 (3.17)	0.3759	0.539
	Above 5 years	137	7 (5.11)		
Breed	Cross bred	58	4 (6.89)	1.091	0.296
	Indigenous	142	5 (3.52)		

observed that, calves were less likely to be reactors to tuberculin test than older animals.

The findings of the present study are not in concordance with Elias *et al*. (2008) who reported that the prevalence of tuberculosis in cattle was more in the age group of 3-6 (43.3%) as compared to in age group above 6 years of age (36.7%) in Ethiopia.

The higher prevalence rate of bovine tuberculosis in older animals may be due to prolonged closed contact. The reason for lower prevalence rate of bovine tuberculosis in young calves may be influence of T-cells, which are predominantly found in the circulation of young calves (Mackay and Hein, 1989).

Breed

In the present study, cattle were divided in 2 breed groups *i.e.,* crossbred and native or indigenous animals. The higher prevalence was observed in crossbred group as 3.45% and 6.89% by SITT and ELISA, respectively; (Table 4) followed by native group as 2.82% and 3.52% by SITT and ELISA, respectively. It indicates that crossbred cattle are more susceptible than native or indigenous cattle for bovine tuberculosis.

The breed wise prevalence (Table 4) was higher in crossbred cattle. The study is in agreement with Didugu *et al.* (2016) who found higher prevalence in crossbred cattle (3.22%) as compared to non-descript cattle (2.08) in Krishna district, Andhra Pradesh, India. These findings are also in agreement with the observations of Bhanurekha *et al*. (2014) who reported higher sero-prevalence in Jersey crosses (9.6%) than indigenous cattle (1.2%) in Tamil Nadu. The present study is also in concordance with Das *et al.* (2017) who reported higher seroprevalence in exotic cross (34.6%) as compared to indigenous cattle (10.5%) in Gangetic delta region of West Bengal, India. Results are also in agreement with the observation of Thakur *et al*. (2010) who observe that prevalence was relatively higher in crossbred cattle than in pure breed cattle in Himachal Pradesh, India.

The findings of present study are not in concordance with Dutta *et al*. (2016) who reported that the prevalence of tuberculosis was more in indigenous breeds than crossbreed cattle in western India. The results also differed to that observed by Mahmud *et al*. (2014) who reported that prevalence of local breed was highest (13.33%) among all crossbreed in Sirajganj district of Bangladesh.

Higher proportion of Holstein cattle in India suffer from advanced diseases since the test-and-slaughter- based control method is not applied in India. Therefore, the disease could progress longer with a greater proportion of animals reaching a more severe disease status (Phaniraja *et al*. 2010). Some other reasons for higher prevalence rate in crossbred cattle may be high production potential of crossbreed animals and birth to a considerable number of young ones. It is directly related to degree of stress on animals. Hence, the animal may be susceptible to active disease (Trangadia *et al.*, 2013). Higher prevalence in crossbred animals might be due to higher disease resistance capability of indigenous cattle.

Statistical analysis

The results were compared using chi-square test. The comparison of results for SITT and ELISA tests was found statistically non-significant ($p > 0.05$) with respect to age and breed (Table 3, 4).

The results are in agreement with Rani *et al*. (2018) who found statistically non-significant ($p > 0.05$) results with respect to age ($p=0.424$) and breed ($p=0.346$). However, the findings of the present study are not in concordance with Rehman *et al*. (2017) who found that animal level prevalence was associated with the age ($÷2=81.14$, $p=0.000$) and breed ($÷2=3.83$, $p=0.003$). The findings of are also not in concordance with Yahyaoui Azami *et al*. (2018) who found that Age higher than 36 months was significantly associated with a higher risk of BTB compared with age below 12 months.

Kappa statistics was used to know the agreement between tests by calculating following parameters.

(i) Observed proportion agreement between the two tests (OP) = 0.955
(ii) Expected proportion of agreement bychance (both positive) (EP+) = 0.00135
(iii) Expected proportion of agreement by chance (both negative) (EP-) = 0.92635
(iv) Expected proportion of agreement by chance (EP) = 0.9277
(v) Observed agreement beyond chance (OA) = 0.0273
(vi) Maximum possible agreement beyond chance (MA) = 0.0723
(vii) *Kappa* (ratio of the OA/MA) = 0.38

On the basis of *Kappa* value (0.38) calculated in this study to know agreement between the two tests, the SIT used in this study was found to be in poor

agreement with ELISA test.

CONCLUSION

Study shows ELISA is a more sensitive test for diagnosis of tuberculosis than the Single Intra Dermal Tuberculin test. Tuberculosis is present in the cattle of Jaipur region, found more in the exotic cattle and the higher age group animals in comparison to the native breeds and lower age group animals. This study confirms the presence of tuberculosis in dairy cattle of Jaipur region. It indicates the higher probability of occurrence of tuberculosis in future. Therefore, there is need to emphasize on the risk factors for preventing potential presence and also design surveillance programs at large scales and highlight the need for a practicable control strategy of this zoonotic disease in the region.

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Conflict of interest

The Authors declare that no conflict of interest exist.

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